

# Detection of Cox2 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

## Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

## Antibody Information:

Blocking Serum: Normal Goat Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog #005-000-001

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog #SP-2001

Primary antibody: Rabbit anti-mouse Cox2

Cayman Chemical

Ann Arbor, MI 48108

[www.caymanchem.com](http://www.caymanchem.com)

1-800-364-9897

Catalog #160106

Negative control serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Concentration: 60 mg/ml

Catalog #011-000-001

Secondary antibody: Biotinylated goat anti-rabbit IgG

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog #BA-1000

Label antibody: Vector Standard Elite Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog #PK-6100

### **Staining Procedure:**

-Positive Control Tissue: Highest level of expression located at the distal vas deferens where it inserts into the bladder. Weak staining at the proximal end of the vas deferens.

-Stain localization: Peri-Nuclear/Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Perform Heat Induced Epitope Retrieval using Microwave Oven.

Place a full rack of slides in container containing 200ml 1X citrate buffer.

Microwave for 5 minutes at power level 5

Cool for 1 minute (Add citrate buffer to container if necessary)

Microwave for 5 minutes at power level 5. Temp after microwaving\_\_\_\_\_

Cool 20 minutes at room temperature

Rinse in distilled water 2 times for 3 minutes each.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Block in 5% Normal Goat Serum for 20 minutes.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply Avidin/Biotin block

Lot# \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

Apply avidin block - 15 min at RT.

Quick rinse in 1X Automation Buffer.

Apply biotin block - 15 min at RT.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Cox2) at 1:1500 dilution and incubate for one hour at room temperature.

Lot# \_\_\_\_\_ Aliquoted yes / no Date Aliquoted \_\_\_\_\_

For the negative control slides, match the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (Cox2), and use this to make the 1:1500 dilution. Apply to the slides and incubate for one hour at room temperature.

Lot # \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary antibody (Biotinylated goat anti-rabbit) at 1:500 dilution and incubate for 30 minutes at room temperature.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Label antibody and incubate for 30 minutes at room temperature.

(Prepare 30 minutes prior to use)

Exp. Date \_\_\_\_\_ New Kit: yes / no

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.

(Add 1 drop of DAB per ml of substrate)

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation Buffer until blue.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

*Updated 08/16/06*